CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

DIAZINON

Chemical Code # 198, SB 950-204, Tolerance # 00153

July 29, 1986
Revised 6/16/88, 2/17/89, 2/27/90, 12/28/93, 8/20/96, 7/21/98, 4/15/99

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, possible adverse effect
Teratogenicity, rat:	No data gap, no adverse effect
Teratogenicity, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, possible adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotoxicity, hen:	No data gap, no adverse effect [1]

[1] Neurotoxicity was seen in an oral, 90-d rat study (record 135600).

Note: Toxicology one-liners are attached. These pages contain summaries only; individual work-sheets may identify additional effects. Volume/record number identifiers in one-liners indicate study status as follows:

** (double asterisks) indicates an acceptable study
Boldface indicates possible adverse effect(s)

Revised filename: T990415
Revised by: Stephen J. Rinkus, 7/21/98; J. Gee, 4/15/99

Available EPA one-liners are included.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC TOXICITY, RAT

153-262 092996 Kirchner, F.R., McCormick, G.C., and Arthur, A.T., "Diazinon (MG-8): One/two-year oral toxicity study in rats". Ciba-Geigy Corp., Summit, NJ, 6/14/91. Diazinon (MG-8), (FL 872049), 87.7% purity, was administered in diets of CD rats at dose levels of 0, 0.1, 1.5, 125, or 250 ppm for 1 yr (10 rats/sex/group) or up to 99 wk (20 rats/sex/group). An additional control group received a stabilizer (which is present in technical diazinon) at a level comparable to that which is present in treated diet at the 250 ppm diazinon level. There were recovery groups of 10/sex/dose for both control treatments and for the 250 ppm level: these were treated for 1 yr and then placed off treatment for 4 wk. Recovery groups were used to evaluate reversal of cholinesterase effects and of possible tissue effects (which were not observed at dose levels employed). NOEL for cholinesterase inhibition = 0.1 ppm (about 50% inhibition of serum cholinesterase in females at 1.5 ppm, generally less inhibition in males). The NOEL for RBC or brain cholinesterase inhibition was 1.5 ppm. Inhibition of brain cholinesterase was 42 and 48% at 99-week study termination in 250 ppm males and females, respectively. NOEL for other effects = 250 ppm (i.e., there were not even clinical signs of cholinesterase inhibition, despite the high level of brain cholinesterase inhibition). The study is **acceptable, although the dosage range was lower than ideal for a chronic study. **No adverse effects**. Aldous, 10/21/92.

153-530 152809 Response by Novartis Crop Protection, NC, dated 2/3/97, to US EPA regarding ophthalmological examinations in the above study, Record No. 092996. The statement regarding that all ocular abnormalities were not examined by the Staff Ophthalmologist referred to findings during clinical exams. All animals were examined pretest and weeks 51 and 97/98. No worksheet. Gee, 4/15/99.

143 039821 "Chronic Feeding (104 weeks) in Rats with Diazinon 25W, Final Report (Includes Interim Progress Reports)." Hazleton Labs, 12/22/55. Test article = Diazinon 25W (nominally 25% active), 0, 10, 100, and 1000 ppm of active ingredient in diet to 20/sex/group except no females at 1000 ppm. No adverse effects indicated. Cholinesterase inhibition NOEL not found (inhibition at LDT of 10 ppm in both sexes). Apparent NOEL for other effects = 1000 ppm (no other effects attributable to treatment). **Unacceptable**, **not upgradeable** (major deficiencies were: too few animals, too few tissues examined, and too much disease.) C. Aldous, 4/4/86.
EPA one-liner: No core grade. ChE NOEL =< 10 ppm.

025, 098, 106 931381 One sentence summary of 039821.

153-106 931319 A two-page summary reporting of toxicological data. No worksheet. (Rinkus, 2/6/90).

CHRONIC TOXICITY, DOG

**153-261 092995 Rudzki, M.W., McCormick, G.C., and Arthur, A.T., "Diazinon (MG-8): 52-week oral toxicity study in dogs". Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ., 6/14/91. Diazinon, FL 872049, 87.7% purity, was fed at initial dietary concentrations of 0, 0.1, 0.5, 150, or 300 ppm to 4 beagles/sex/dose level. After 14 wk, the diet for the 300 ppm group was reduced to 225 ppm for the balance of the study, due to marked b.w. gain decrements. Mean dose levels were 0.0032, 0.015, 4.7, and 7.7 mg/kg/day for males; and 0.0037, 0.020, 4.5, and 9.1 mg/kg/day for females. NOEL (other than cholinesterase inhibition) = 0.5 ppm (decreased b.w. in 150 ppm males

and in 300 ! 225 ppm males and females: decreased food consumption in both sexes at 150 and 300 ! 225 ppm). Cholinesterase inhibition NOEL = 0.1 ppm in males and marginally < 0.1 ppm in females (statistically significant reductions in serum cholinesterase activity compared to concurrent controls at two out of four intervals in females at 0.1 ppm). At 150 and 300 ! 225 ppm, serum and RBC cholinesterase activities were significantly reduced in both sexes, and brain cholinesterase activity was significantly reduced in females. The lack of a definitive NOEL for serum cholinesterase is not a pivotal issue in this study, since brain and RBC cholinesterase inhibition effects were limited to much higher doses, and none were accompanied by definitive treatment effects, even at the highest dose levels. A few other findings, typically limited to the 300 ! 225 ppm group, suggested possible treatment effects. The most notable of these was elevated serum amylase activity at several time points at 150 ppm and above in both sexes (no corresponding histopathology at termination). **Acceptable, with no adverse effects**. Aldous, 7/27/93.

143 039820 and 137 026882 "Chronic Dog Feeding Study (46 Weeks) in Dogs with Diazinon, Wettable Powder, Final Report (Includes Interim Progress Reports)." Hazleton Labs, 10/20/54. Diazinon, approx. 23% formulation given by capsule, frequency and quantity of administration varied. Reported dosages (when initially administered) = 0, 4.6, 9.3, and 23.1 mg/kg/day. Apparent NOEL = 4.6 mg/kg/day (cholinergic effects at higher doses required occasional interruption of treatment). **Unacceptable.** Deficiencies include: only 2 dogs/treatment, inappropriate test article, sporadic treatment protocol, etc. No further information required on this study. A. Apostolou, 9/05/85, C. Aldous 4/4/86.
EPA one-liner: No core grade. ChE NOEL = 4.6 mg/kg.

CHRONIC TOXICITY, MONKEY

143 039822 "Diazinon 50W- Safety Evaluation by Repeated Oral Administration to Monkeys for 106 Weeks-Final Report." Woodard Research Corp., 6/6/66. Diazinon 50W formulation administered at 0, 0.05, 0.5, and 5.0 mg/kg/day, 6 days/wk, by gavage tube to 3/sex/group, Rhesus monkeys. Cholinesterase enzyme inhibition NOEL = NOEL for clinical observations (soft stools) = 0.05 mg/kg/day. Possible weight gain decrement in females at 5.0 mg/kg/day. **Unacceptable**, **not upgradeable**: inappropriate dosing material, too few animals, and limited individual data.
C. Aldous, 4/4/86.
EPA one-liner: No core grade. ChE NOEL = 0.05 mg/kg, 14-25% plasma ChE inhibition at 0.5 mg/kg. No effects seen on hemograms, blood chemistry and body weights.

025, 098, 106 931957 One sentence summary of 039822.

099 931440 Brief summary of 143:039822, above.

153-530 152809 Response of Novartis Crop Protection to US EPA regarding analytical chemistry data for the above study, Record No. 039822. No data for the dosing preparations are available. No worksheet. Gee, 4/15/99.

122-032 031883 "Pilot Study of the Effects of Pesticides on Blood Lipoproteins, Arteries, and Cardiac Muscle of Baboons" (H.C. McGill [author], EPA contract No. 68-01-1914). This document per se could not be located in the CDFA Library, but it appears to be identical to 122-038 056781, which concerns a 2-yr feeding study using baboons of both sexes. The following were tested separately: Diazinon, Chlordane, Parathion, and Carbofuran. Diazinon was tested at 0, 0.1, and 0.4 mg/kg/day. No

toxicological effects could be attributed to the Diazinon treatments. Supple-mental information. No

worksheet. (Rinkus, 2/6/90).

ONCOGENICITY, RAT

027, 121, & 144 931506 "Bioassay of Diazinon for possible carcinogenicity" Gulf South Re-search Institute, 1979. Diazinon, purity not given, from Ciba-Geigy Lot No. Fl-741306. Doses of 0, 400, and 800 ppm in feed to 50/sex Fischer F344 rats in treated groups and 25/sex in concurrent controls. **No neoplasia, and no adverse effects indicated. First CDFA review by J. Wong (5/23/85) found study not acceptable due to lack of sufficient dose justification, control group appeared too small, and other deviations from guidelines characteristic of NCI studies. Reevaluation by C. Aldous (6/10/88) prompted by rebuttal comments in 153-165 (no record number) reclassified study as **acceptable**, for reasons given in 6/10/88 review (dose is justified based on cholinesterase inhibition; small control group sizes are compensated by high survival in concurrent controls and availability of near-concurrent controls from other studies performed at about the same time at same facility; some of the deviations from guidelines identified in the 1985 review are chronic study requirements, which are not critical for oncogenicity studies, and an additional chronic study is now required by EPA). C. Aldous, 6/16/88.

ONCOGENICITY, MOUSE

027, 121, & 144 931507 "Bioassay of Diazinon for possible carcinogenicity" Gulf South Re-search Institute, 1979. Diazinon, purity not given, from Ciba-Geigy Lot No. Fl-741306. Doses of 0, 100, and 200 ppm in feed to 50/sex B6C3F1 hybrid mice in treated groups and 25/sex in concur-rent controls. **No neoplasia, and no adverse effects indicated. First CDFA review by J. Wong (5/23/85) found study not acceptable due to lack of sufficient dose justification, control group appeared too small, and other deviations from guidelines characteristic of NCI studies. Reevaluation by C. Aldous (6/16/88) prompted by rebuttal comments in 153-165 (no record number) reclassified study as not acceptable, but upgradeable on receipt of dose justification, plus certain historical control data. These data were provided in Vol. 196, Records 085219-085221 (see below). Study is **acceptable**. C. Aldous, 6/16/88, 11/8/89.

153-196 085219 "4-week feeding study in mice". Ciba-Geigy Corp., Div. of Toxicology/Pathology, Summit, N.J., 9/28/89. Male and female CD-1 mice were dosed with 0 or 200 ppm diazinon for duration of 2 or 4 weeks (20/sex/exposure duration). Test article (FL 872049, 87.7%) was pre-pared in diet, adjusted for purity. Parameters measured included mortality, clinical signs, body weight, food consumption, physical examinations (pre-dosing, and 1 day prior to necropsy), limited hematology, limited general blood chemistry, and cholinesterase (ChE) assays of RBCs, plasma, and brain. Results: no effects on mortality, clinical signs, body weight, or food consumption. There were too few samples to statistically evaluate hematology and general blood chemistry measurements, however no changes were apparent. ChE effects were substantial: RBC ChE inhibition was 44-48% in males, 58-61% in females. Plasma ChE inhibition was 97% in males, and 98% in females. Brain ChE inhibition at days 16 and 30, respectively, was 28 and 38% for males, and 21 and 37% for females. Data are consistent with widely accepted criteria for acceptability of dosage range in an oncogenicity study. C. Aldous, 11/06/89.

153-196 085220 and 085221 (Eleven pages <u>preceding</u> 085220 contains a non-titled memo from Jerry F. Hardesty, D.V.M., Pathologist at Experimental Pathology Laboratories, Inc. to Dr. William Campbell of Ciba-Geigy Corp., plus a C.V. of Dr. Hardesty. Record 085220 contains a chapter, "Tumours of the mammary gland" by F. Squartini, from the book, <u>Pathology of tumours in laboratory animals, Vol. II, tumours of the mouse</u>, IARC Scientific Publications No. 23, Lyon, 1979. Re-cord

085221 contains a chapter, "Selection and use of the B6C3F1 mouse and F344 rat in long-term bioassays for carcinogenicity" by D.G. Goodman, G.A. Boorman, and J.D. Strandberg, from the book, Handbook of carcinogen testing, H.A. Milman and E.K. Weisburger, Eds., Park Ridge, NJ, Noyes Publications, 1985. The memo from Dr. Hardesty was dated 9/7/89. Data presented were sufficient to demonstrate that these two tumor types were not meaningfully increased in incidence with dose. This information, in addition to the 4-week feeding study simultaneously submitted (Record No. 085219) permit an upgrade of the mouse oncogenicity study, 027:931507 to **acceptable** status, with **no adverse effect**. See review by C. Aldous, 11/8/89.

REPRODUCTION, RAT

137, 145 026870 "Diazinon-Three Generation Reproduction Study in Rat (50% Wettable Powder)." Woodard, 1965. Diazinon fed to 20/sex at 0 or 4 ppm for F0 generation, then next two generations (10 males/20 females per generation) received 0, 4, or 8 ppm: two litters/generation. **Unacceptable.** No adverse effects. This study cannot be upgraded as there were only 2 dose levels, clearly unjustifiable with respect to an MTD. A. Apostolou, 9-5-85.
EPA one-liner: No core grade. 3 generation NOEL = 4 ppm (only dose tested). 2 generation NOEL = 8 ppm (two dose tested)

137 026871 Publication. Insufficient information and design deficiency preclude evaluation for adverse effects. Unusually low body weights suggest husbandry problems and absence of concurrent controls make these values worthless. No adverse effect noted. A. Apostolou, 9-5-85, J. Parker and F. Martz, 3-31-86.

****153-189 073162** "A Two Generation Reproductive Study in Albino Rats" (Raab et al.; Ciba-Geigy Corp.; Toxicol./Pathol. Report 88128; 2/9/89). Diazinon, purity of 95%, was presented in the feed at concentrations of 0, 10, 100, and 500 ppm, which were verified analytically. F0 rats and F1 rats, derived from F1a litters, were exposed for 10-11 weeks before their single mating trials and were exposed for a total of 16-21 weeks before they were sacrificed. F0 parental effects were limited to the high-dose group and included: tremors during the postmating phase in 3 dams and lower gestational BW gains and lactational BWs. F1 high-dose males and dams weighed respectively 26% and 16% less than did the controls after weaning; males still weighed 15% less at mating and 14% less at termination; females weighed 6% less at mating but had BWs equal to those of the controls at termination. F1 mid-dose males weighed less and had gained less weight than the controls at mating. F1 dams weighed less than the controls at the start of their gestations and gained less weight during their gestational and lactational periods. Tremors also were seen in 4 F1 high-dose dams during their postmating phase. Fertility was not affected in an unequivocal manner in this study, but both F0 and F1 high-dose groups exhibited increased incidences of prolonged gestations ($\geq$ 24 d). Progeny effects included: decreased live litter size for the high-dose F2a litters and decreased pup survival and pup BWs during lactation for the F1a mid- and high-dose groups and for the F2a high-dose group. **Progeny NOEL = 10 ppm (decreased pup survival and post partum BWs).** When first reviewed (Rinkus, 2/1/90), this study was considered unacceptable but upgradable upon submission of: 1) greater explanation of procedure and results for pup necropsies; and 2) justification for designating the mammary glands as the target organ. The Registrant responded by submitting records 092536, 095071 and 112990. Record 092536 is discussed in worksheet W092536.834 and the other two records are discussed in worksheet W073162.S01. This study now is considered **ACCEPTABLE** but the following new findings have been made: 1) the tremors appear to be the result of birthing problems, as opposed to organophosphate neurotoxicity; 2) the incidence of total litter resorptions was increased in each of the Diazinon-treated groups in the F2a trial; and 3) upon recalculation, the fertility index for the F2a trial was not de-creased; rather, the corresponding gestation index was decreased for each of the Diazinon-treat-ed groups (**reproductive NOEL < 10**

ppm). (Rinkus, 12/27/93).

153-224 095071 (Supplementary data to 153-189:073162). Sova, J.A., and Breckenridge, C., "Additional data requested by the California Department of Food and Agriculture to upgrade the Diazinon Technical two-generation reproductive study in rats". Date of additional data: July 13, 1990. This submission provided summaries of F. and F. pup disposition data (including numbers of pups necropsied at day 4 cull and at postweaning termination). Also, gross necropsy records for day 4 cull and at postweaning pups were presented. A log of "descriptive notes" which included abnormal necropsy findings of pups dying on study was provided. Aldous, 9/23/92.

153-265 112990 (Supplementary data to 153-189:073162). Sova, J. and Breckenridge, C., "Second response to upgrade the two generation reproduction study in albino rats", Jan. 31, 1992 (date of present submission). The submission addresses the major issues raised in the CDFA review of Feb. 1, 1990. Major new data in this submission include: (1) summary tables of fates of pups (accounting for numbers of pups stillborn, found dead, or missing (and presumed cannibalized), (2) confirmation that all dead pups (except those cannibalized) were grossly examined, (3) an explanation of the extent of gross examinations, (4) explanation why mammary gland was examined as a possible "target organ", and (5) appendices showing dispositions of individual litters over time. A number of additional concerns brought up in the original CDFA review were addressed. Aldous, 9/23/92.

153-225 092536 "A One Generation (Two Litter) Reproductive Study in Rats" (Raab et al.; Ciba-Geigy Corp.; Toxicol./Pathol. Report 88055 (MIN 842294); 1/25/89). Diazinon, purity of 95%, was presented in the feed at concentrations of 0, 10, 100, and 1000 ppm, which were verified analytically. Parental rats were exposed for 13 and 26 weeks before their first and second mating trials, respectively, and were exposed for a total of 29-31 weeks before they were sacrificed. Exposure to Diazinon affected the reproductive performance of the rats in a manner that was dependent on dose as well as duration of exposure. Fecundity (# live deliveries/# cohoused) was decreased at the 1000 ppm treatment level for the F1a mating trial, and at the 10 ppm, 100 ppm and 1000 ppm treatment levels for the F1b mating trial. Whether the fecundity decreases involved decreases in mating, fertility and/or gestation indices could not be discerned from the report due to uncertainty over whether the number of dams that mated and the number that became pregnant had been determined accurately. This appears to be caused by the occurrence of total litter resorptions in the Diazinon-treated groups, indicated by large bodyweight gains after mating, which were not followed by deliveries. **Reproductive NOAEL < 10 ppm (decreased fecundity in the F1b trial).** Parental effects included: prolonged durations of gestation followed in some cases by postbirthing tremors in the 1000 ppm dams; decreased gestational and lactational bodyweights and/or bodyweight gains in the 1000 ppm dams; and reduced absolute ovary weights and/or ovary weights relative to bodyweight in the 100 ppm and 1000 ppm dams. **Parental NOAEL = 10 ppm (reduced ovary weights).** Progeny effects included: decreased live litter sizes (each of the Diazinon-treated groups, both trials); increased incidences of stillbirths (1000 ppm group, both trials; 10 ppm group, F1b trial); and decreased pup survival and pup BWs during lactation (100 ppm and 1000 ppm groups, both trials). **Progeny NOAEL < 10 ppm (decreased live litter size, both trials).** Although this study is **UNACCEPTABLE AND NOT UPGRADABLE** (not a FIFRA guideline study), this study has provided important information about Diazinon's effects on reproduction. However, a more thorough analysis of this study would require the following information: 1) all bodyweight measurements made after cohousing started for those dams not observed to be sperm positive in a mating trial; 2) uterine implantation data and corpora lutea data for all dams in the study; 3) individual pathology data (histology and/or necropsy) for all parental rats; 4) F1a litter data

for dams 3510 and 3513; 5) detailed historical control data regarding F1a and F1b litters; and 6) the individual data for the cholinesterase testing. **Supplementary information.** (Rinkus, 12/27/93).

TERATOLOGY, RAT

137 026874 Publication summary. A. Apostolou, 9-5-85, Insufficient information to evaluate. NOTE by C. Aldous (6/14/88): Doses were ip, and report claims that "tolerated" dose of 150 mg/kg increased resorptions. This dose was about half of a lethal dose, hence probably was maternally toxic. A 100 mg/kg dose did not appear to be fetotoxic. There were only 4-6 dams/dose, hence study was extremely limited. CNA agrees with AA that there is not sufficient information for evaluation, and agrees that the reported resorptions do not indicate meaningful evidence of a "possible adverse effect".

137, 039, 148 026875 "Reproduction Study - G 24480 (Diazinon Technical) Rat, Segment II (Test for Teratogenic or Embryotoxic Effects)." (Ciba-Geigy, 1974) **Unacceptable.** Technical diazinon (no purity stated) given by oral gavage at 0, 15, 50, or 100 mg/kg/day, days 6-15, to 30 Sprague-Dawley rats per group. No adverse effect. No individual data are presented. J. Wong, 5-23-85 and A. Apostolou, 9-5-85.
EPA One-liner: NOEL = 100 mg/kg (HDT). Core grade = Supplementary.

153-149 039836 "Teratology Study (Segment II) in Albino Rats with Diazinon Technical." (Ciba Geigy, 4/19/85) Diazinon technical, no purity stated, was administered to Crl.COBS CD (SD) (BR) rats by gavage on day 6 through 15 of gestation at 0, 10, 20 or 100 mg/kg/day to 27/group. No adverse effects indicated. Slight maternal toxicity (weight gain and food consumption) and developmental toxicity (structural changes) seen at 100 mg/kg/day. Initially reviewed by Parker, 3-13-86 as unacceptable, upgradeable. Need to supply analytical data for dosing suspensions, dam necropsy data. No adverse effect noted. NOEL for maternal and developmental toxicity = 20 mg/kg/day. Dam necropsy data and dose solution analysis were supplied in document 153-183/ record 070885 and the purity of the test material, 97.4%, was supplied in document 153-193/re-cord 075062. Study is upgraded to **ACCEPTABLE. (Parker 11/10/88; Gee & Rinkus, 2/16/89; Rinkus, 12/14/89).

183 070885 Supplemental data to 149 039836. Analytical and necropsy data. (Kishiyama and Parker, 11/10/88).

153-193 075062 Supplemental data to 153-149 039836. Purity of test material used in the rat teratology study. (Rinkus, 12/14/89).

TERATOLOGY, RABBIT

150 039837 (pilot) and 039838 "Teratology Study of Diazinon (CAS Number 333-41-5) in New Zealand White Rabbits," (Science Applications, Inc., 7/28/81) Diazinon, 89.2%, was administered to New Zealand White rabbits by gavage on days 6 through 18 of gestation at 0, 7.0, 25.0 or 100 mg/kg/day; 19-22/group. Maternal NOEL =25 mg/kg/day: mortality, 9/22, and increased clinical signs at 100 mg/kg/day. Developmental toxicity NOEL = 100 mg/kg/day, no effect on fetal parameters at any dose level. Initially reviewed by Parker, 3-13-86, as unacceptable, no adverse effects. Need to supply analysis of dosing suspensions, dam necropsy data and body weight data for all days. Necropsy, body weight, analytical data were submitted and considered adequate (document 183/record 070884). Study is upgraded to **ACCEPTABLE. (Parker, 11/10/88; Gee & Rinkus, 2/16/89).
EPA one-liner: Mimimum. Maternal NOEL = 25 mg/kg, increased mortality; Fetal toxicity NOEL = 100 mg/kg (HDT); Teratogenic NOEL > 100 mg/kg (HDT)

183 070884 Supplemental data to 150-039837 (pilot) and 039838. Necropsy, body weight, analytical data. (Kishiyama and Parker, 11/10/88).

153-530 152809 Response of Novartis Crop Protection to US EPA regarding the day of onset of clinical signs and of death at the high dose in Record No. 039837. The individual animal data were provided regarding day of death and day of observations for those which died during the study and those which survived to termination. No worksheet. Gee, 4/15/99.

TERATOLOGY, OTHER SPECIES

137 026872 Publication summary. Chick, insufficient information to evaluate.
A. Apostolou, 9-5-85.

137, 147 026873 Publication summary. Hamster and rabbit, insufficient information to evaluate. A. Apostolou, 9-5-85.

GENE MUTATION

Summary. Two acceptable submissions in this category are records 074159 and 089069, using bacterial test systems. No induction of mutations was observed. The positive results in record 089071 would suggest that Diazinon degradation products may be genotoxic. This data requirement is considered satisfied. (Gee, 10/2/92).

** 229 089069 "Gene Mutation Test; Salmonella and Escherichia/Liver-Microsome Test" (D. Geleick, Ciba-Geigy, Basle, Switzerland, 2/8/90, Study Number 891346) Diazinon technical, 88.0% purity, was tested with Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 and Escherichia coli strain WP2uvrA, with and without rat liver activation. Concentrations per plate were 0 (DMSO), 313, 625, 1250, 2500 or 5000 Fg with triplicate plates per concentration, two trials. No increase in revertants was reported. **Acceptable.** No adverse effect. Gee, 10/1/92.

229 089071 "Responses of the L5178Y tk+/tk- Mouse Lymphoma Cell Forward Mutation Assay: III. 72 Coded Chemicals" (McGregor, D. B. et al., Environmental and Molecular Mutagenesis 12: 85-154 (1988) Diazinon was tested twice with mouse lymphoma cells without S9 activation. Concentrations were 0 (DMSO), 6.25, 12.5, 25, 50, 100 and 200 Fg/ml in trial 1 and 0, 20, 40, 60, 80 or 100 Fg/ml in trial 2. MMS was the positive control. An increase in mutation frequency was found at 100 Fg/ml in trial 1 and at 60 and 80 Fg/ml in trial 2. The highest concentration in each trial was "lethal". MMS was not effective as a control in the second trial. Comments prefacing the publication suggest that, since the material used was produced in 1974 and was at least 10 years old, the positive results could be due to impurities or breakdown products. The data reported indicate a positive result. **Unacceptable**, not upgradeable. Gee, 10/1/92.

137, 146, 176 026878 "Screening of Pesticides for Mutagenic Potential Using Salmonella typhimurium Mutants," (Marshall et al., J. Agric. Food Chem., 24: 560-563, 1976). Diazinon, probably not technical grade (test material was described as a pure form from stocks of "standards used in residue analysis"), was tested for mutagenicity in the Ames test at 1000 Fg/plate, without and with phenobarbital-induced rat liver S-9, using TA-strains 1535, 1536, 1537, and 1538. No adverse effect was noted. Unacceptable because the test material was not tested up to a toxic level and TA-strains 100 and 98 were not part of the screening. {Note: document 137 contains only the first two pages of this article, which is reported in its entirety in documents 146 and 176} (Rinkus, 12/28/88).

137, 146, 176 026879 Diazinon was tested for mutagenicity using E. coli in the paper-disk method; no inhibition zone and no mutant colonies were observed. Unacceptable because the disk method

is not useful with insoluble agents like Diazinon; also, no metabolic activation system was employed. No worksheet. (Rinkus, 12/28/88).

137, 146, 176 039828 "Comparative Mutagenicity Studies with Pesticides," (In: IARC Publication No. 10, R. Montesano et al. (eds.), 1974, pp. 161-181). Diazinon, purity and treatment levels not stated, was reported not to be active in some microbial assays that measured gene mutation or mitotic recombination. Unacceptable because the testing did not use any metabolic activation system and, in general, the reporting is insufficient. No worksheet. (Rinkus, 12/28/88).

178, 229 068557, 089070 "Diazinon--Gene Mutation Test: L5178Y/TK+/- Mouse Lymphoma Mutagenicity Test" (P. Dollenmeier, Ciba-Geigy Limited, Basle, Switzerland; Lab. study no, 840396, 7/31/86 and 9/26/90) G 24480 technical (Diazinon). 97.2% purity, without S9 activation at concentrations of 0 (1% DMSO as the final concentration in the cell media), 12, 24, 48, 72, 96, 108 and 120 Fg/ml and with S9 activation at concentrations of 0, 6, 12, 24, 36, 48, 54 and 60 Fg/ml, was tested for mutagenic effects on L5178YTK+/- mouse lymphoma cells in vitro. Highest treatment levels resulted in severe cytotoxicity. No mutagenic effect was observed in testing at the other treatment levels, without or with S9 activation. Results with DMN (8083 Fg/ml) indicated an inadequate S9 system. Reviewed as unacceptable but upgradeable with submission of evidence that the S9 system could activate known promutagens with an efficiency comparable to that reported in the literature. (Rinkus, 12/9/88) Record 089070 addresses the S9 issue, comparing mutation frequencies of 2-AAF and 3-MCA with 10% or 1% S9 from the open literature. Re-review finds there was a single trial. The study is **unacceptable** and not upgradeable. Gee, 10/1/92.

176 067754 (Poole et al., Toxicol. Appl. Pharmacol., 44: 196, 1977.) Abstract on microbial genotoxicity testing (five assays) of 14 pesticides, including Diazinon; testing included the Ames test with TA-strains 1535, 1537, 1538, 98, and 100, and the use of some metabolic activation system derived from the livers of Aroclor-induced rats. Testing was conducted as part of **EPA**'s substitute pesticide program. No adverse effect noted. Unacceptable because of insufficient reporting. No worksheet. (Rinkus, 12/28/88).

176 067818 (Mohn, G., Mutation Research, 21: 196, 1973) Abstract, the full reporting of which is apparently Record 067843. (Rinkus, 12/28/88).

176 067843 "5-Methyltryptophan Resistence Mutations in Escherichia coli K-12: Mutagenic Activity of Monofunctional Alkylating Agents Including Organophosphorus Insecticides," (Mohn, G., Mutation Research, 20: 7-15, 1973), Diazinon, purity not stated, was tested at 13 mM (3957 Fg/ml) for its ability to induce mutations in E. coli K-12/galR^{s} that confer resistance to 5-methyltryptophan. No adverse effect. Unacceptable because the testing did not include the use of any metabolic activation system; also, it is questionable whether Diazinon, which has a water solubility of only 40 Fg/ml, could be tested at 3957 Fg/ml. (Rinkus, 12/28/88).

176 068528 "An Overview of Short-term Tests for the Mutagenic and Carcinogenic Potential of Pesticides," (Waters et al., J. Environ. Sci. Health, B15: 867-906, 1980). Review of an **EPA program** on genotoxicity testing of pesticides, including technical grade Diazinon. Critical experimental details (e.g., methods, treatment levels, and controls) were not stated. Diazinon was reported to be negative in the following testing, in the absence and presence of some metabolic activation system: gene mutation in S. typhimurium (TA-strains 1535, 1537, 1538, 100) and in E. coli WP2; mitotic recombination in S. cerevisiae; differential toxicity in bacteria (Pol A assay and Rec assay); and unscheduled DNA synthesis in WI-38 cells. Unacceptable because of insufficient reporting. No worksheet. (Rinkus, 12/29/88).

**153-192 074159 "In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of

Eighteen Pesticides (Ames Test Results)," (Simmon, V., NTIS document PB80-133226). This is the EPA-sponsored study mentioned in records 067754 and 068528. Diazinon, technical grade (batch FL-741305; purity not stated), was tested for mutagenicity in the Ames test, using strains TA1535, TA100, TA1538, TA98, and TA1537, in the absence and presence of a metabolic activation system (S-9 made from Aroclor 1254-induced male SD rat liver). Test concentrations were: 0 (presumably, DMSO), 1, 10, 50, 100, 500, 1000 Fg/plate in the first trial; and 0, 10, 50, 100, 500, 1000, and 5000 Fg/plate in the second trial. Deficiencies in the report include the lack of reporting on some experimental details and the use of only single plating for each concentration tested per trial. However, based on the facts that the results for the negative and positive controls look satisfactory in each trial, two independent trials were conducted, neither trial gave any indication of a positive response, and concentrations up to 5000 Fg/plate were tested, with and without a proven metabolic activation system, CDFA feels it can accept the study, with no adverse effect indicated. (Rinkus, 1/17/90).

153-192 074159 "In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides (E. coli WP2 uvrA Results)," (Simmon, V., NTIS document PB80-133226). This is the EPA-sponsored study mentioned in records 067754 and 068528. Diazinon, technical grade (batch FL-741305; purity not stated), was tested for mutagenicity in the E. coli tryptophan reversion assay, using only strain WP2 uvrA, in the absence and presence of a metabolic activation system (S-9 made from Aroclor 1254-induced male SD rat liver). Test concentrations were: 0 (presumably, DMSO), 1, 10, 50, 100, 500, 1000 Fg/plate in the first trial; and 0, 10, 50, 100, 500, 1000, and 5000 Fg/plate in the second trial. Deficiencies in the report include the lack of reporting on some experimental details, the use of only single plating for each concentration tested per trial, and the use of only one of the three strains standardly used in this assay. Since one of the missing strains was the one containing the plasmid (WP2 uvrA/pKM101), the testing has to be considered incomplete. In which case, the study is UNACCEPTABLE, and not upgradable, with no adverse effect indicated. (Rinkus, 1/17/90).

CHROMOSOME EFFECTS

Summary. Of the many submissions in this category, only two were considered acceptable. Record 068510 indicates that human lymphocytes in S-phase were affected by a 1-h treatment with Diazinon. Record 068499 indicates that Diazinon, even when tested up to toxicity, did not cause chromosomal damage of the type that can be detected in the bone-marrow micronucleus test with mice. For the purposes of hazard identification, this data requirement is considered satisfied, with an adverse effect indicated, based on the in vitro study. (Rinkus, 1/5/89).

137, 146 026881; 176 068516 "Dominant Lethal Study on G 24480 (Diazinon Technical.) Mouse (Test for Cytotoxic or Mutagenic Effects on Male Germinal Cells)." (Ciba-Geigy, 1975) Diazinon, no description; 12 males/group, single dose of 0, 15 or 45 mg/kg by oral gavage; mated 1:3 for 6 weeks; No adverse effect; NOEL > 45 mg/kg. **Unacceptable.** Missing info, no positive control. A. Apostolou, 9-5-85, J. Gee, 3-13-86. Record 68516 is identical to record 26881, except that it has a supplemental section, dated 5/26/87. The test material now is described as a batch produced in 1967 for which there are no analytical data; however, it should be like other batches produced in that period, in which case it would have a purity of about 95%. The HOT is said to have been selected to be 1/3 of the LD_{50}. While not done concurrently, the results from testing Trenimon at two levels in that same year of the Diazinon study (1975?) are now presented as "positive controls." Still unacceptable because an MTD in NMRI mice is about 120 mg/kg according to record 68499, in which case the HOT that was used is considerably less than the M.D. No worksheet. (Rinkus, 1/3/89).

**178 068499, "Diazinon--Structural Chromosomal Aberration Test: Micronucleus Test, Mouse", (Ciba-Geigy Limited, Base, Switzerland; Lab. study no. 871696, 5/20/88). G 24480 technical (Diazinon), 87.5% purity, was tested for the induction of micronuclei in bone-marrow polychromatic erythrocytes (PCEs) of NMRI mice. Testing was done in two parts. In part I, 5 mice/sex/sacrifice time were gavaged once with an aqueous suspension of Diazinon at 120 mg/kg and then were sacrificed 16, 24, or 48 hours later. In part II, 5 mice/sex/treatment level were gavaged once with 0 (water), 30, 60, or 120 mg/kg and then were sacrificed 24 hours later. In both parts, there was no induction of micronucleated PCEs even though the HDTs did result in some mortalities (1 death in 24 males in part I and 2 deaths in 8 males in part II). No adverse effect. ACCEPTABLE. (JSK, SJR, 12/13/88).

178 068556, "Diazinon--Structural Chromosomal Aberration Test: Nucleus Anomaly Test in Somatic Interphase Nuclei", (Ciba-Geigy Limited; Base, Switzerland, Lab. study no. 801503, 11/5/81). G 24480 (Diazinon), purity not stated, was given twice by gavage 24 hours apart at 0 (PEG 400), 6.5, 13 and 26 mg/kg/day to Chinese Hamsters 3-6/sex/group, with sacrifice 24 hours after the second treatment. Assay consisted of scoring a total of 1000 bone-marrow cells (leukocytes, erythroblasts as well as PCEs) for presence of micronuclei. No adverse effects noted. **UNACCEPTABLE**, may be upgraded with submission of: (1) purity of test material; (2) LD50 data; and (3) further evaluation of the slides for the frequency of micronuclei in one cell type, based on the analysis of >500 cells/animal, with 5/group; or better documentation that the assay is more sensitive than the standard micronucleus procedure (JSK, SJR, 12/13/88).

176 068507 "Chromosomal Aberration Tests on 29 Chemicals Combined with S9 Mix In Vitro," (Matsuoka et al., Mutation Research, 66: 277-290, 1979). Diazinon (purity not stated) was tested only at 100 Fg/ml, without and with aroclor-induced, rat-liver S-9, for its ability to cause chromosomal aberrations in Chinese hamster lung cells of an established cell line. The final concentration of S-9 fraction in the cell solution during the 3 h treatment period was 21% (v/v). Without S-9, severe cytotoxicity prevented any analysis; but, with S-9, the incidence of chromosomal aberrations (gaps, breaks, but predominantly exchanges) was 27%. While no actual negative controls were reported, the results with inactive test materials would indicate that the corresponding control frequency is $\leq$ 3.5%. Unacceptable because a dose response and survival data are needed to distinguish between the possible metabolic activation of Diazinon versus a manifestation of the cytotoxicity, which is presumably still present but lessened in the presence of the S-9. (Rinkus, 12/29/88).

176 068508 "Influence of Diazinon and Lindane on the Mitotic Activity and the Caryotype of Human Lymphocytes, Cultivated in vitro," (Tzoneva-Maneva et al., Bibliotheca. Haematologica.[Basil], 38: 344-347, 1971). Diazinon (purity not stated) was tested up to 5 Fg/ml for its ability to induce chromosome aberrations in cultured human peripheral blood lymphocytes. Decreased mitotic index and increased incidence of chromosome damage were alleged. Unacceptable because of insufficient reporting, including no table of results. No worksheet. (Rinkus, 12/29/88).

176 068509 "In Vitro Induction of Alterations in Peripheral Blood Lymphocytes by Different Doses of Diazinon," (Lopez et al., Bull. Environ. Contam. Toxicol., 37: 517-522, 1986). Diazinon (purity not stated, but identity was confirmed by NMR, IR and UV) was tested at 0 (2.1% DMSO as final concentration in cell medium), 5, 10, 20 and 30 Fg/ml for its ability to induce chromosome aberrations in cultured human peripheral blood lymphocytes. In a subsequent study (Record 68510), it is stated that the cells in this study were treated for the entire culture period of 72 h. The mitotic index and the frequency of metaphases with chromosome aberrations decreased with increasing dose (negative dose response). The percent of metaphases with decondensed chromosomes increased with increasing dose: at 20 and 30 Fg/ml, the values were increased by a factor of 13 over the DMSO control. Unacceptable because no metabolic activation system was used. [This entire article appears to have been published previously in Spanish in Genet. Iber., 37: 193-200, 1985.] No worksheet. (Rinkus, 12/29/88)

****153-176 068510** "Sensitivity of Human Lymphocyte Chromosome to Diazinon at Different Times During Cell Culture," (Lopez, D.E. and Carrascal, E., Bull. Environ. Contam. Toxicol., 38: 125-130, 1987). Diazinon (purity not stated but presumably the same batch used in Record 68509) was tested only at 30 Fg/ml for its ability to induce chromosome aberrations in cultured human peripheral blood lymphocytes in so-called pulse testing. Cells were treated with Diazinon for only 1 h; the time that Diazinon was added to the 72-h cultures was compared at 6-h intervals: 0, 6, 12, 18,...66 h. When Diazinon was added at 36th hour of culturing (and then removed 1 h later), the percent of metaphases with chromosome aberrations and the percent of metaphases with decondensed chromosomes increased to 26.6% and 14.2%, respectively. Pulse testing with DMSO (the solvent) was not done, but the values for 72 h treatments with DMSO alone (apparently those ob-served in Record 68509, in which case 2.1% (v/v) was the final DMSO concentration) were 8.3% and 3.6%, respectively. In the 12/30/88 review, this study was considered to be "acceptable" and to indicate that cytotoxicity masks the chromosomal effects and that Diazinon caused a chromosomal damage because it seems improbable that DMSO alone tested as a one-hour pulse any time during a 72 h culture period would cause >25% of the cells to have chromosome damage. Record 085204 asked that this study be downgraded because of the apparent lack of a concurrent control and because the experimental details were described incompletely. CDFA recognizes that there were shortcomings in the reporting of this study, but the assignment of an adverse effect will only be changed by submitting a replacement human lymphocyte cytogenetic study, in which no adverse effect is seen. Until then, this study is considered acceptable and the data requirement of chromosomal studies is considered satisfied, with an adverse effect indicated, based on this study. (Rinkus, 12/15/89).

176 068511 "Chromosome Aberrations in Patients Suffering Acute Organic Phosphate Insecticide Intoxication," (Van Bao et al., Humangenetik, 24: 33-57, 1974). Report includes a cytogenetic study of blood lymphocytes taken from a 30-year-old male after he attempted suicide by ingesting some Diazinon-containing product described only as Basudin E (Hungary). No adverse effect was noted. Supplemental information. No worksheet. (Rinkus, 12/30/88).

176 068512 "Lymphocyte Chromosome Analysis of Agricultural Workers during Extensive Occupational Exposure to Pesticides," (Yonder et al., Mutation Research, 21: 335-340, 1973). Cytogenetic analysis of blood lymphocyte cultures taken from pesticide applicators with exposure to multiple agents, including Diazinon. Supplemental information. No worksheet. (Rinkus, 12/30/88).

176 068513 "Chromosome Studies in Workers Producing Organophosphate Insecticides," (Kiraly et al., Arch. Environm. Contamin. Toxicol., 8: 309-319, 1979). Workers at the Budapest Chemical Works producing Basudin E (Diazinon) exhibited an increased frequency of chromatid aberrations and of stable chromosome-type aberrations in comparison to two control groups (nonfactory controls and factory workers not exposed directly to pesticides, e.g., office staff). Supplemental information. No worksheet. (Rinkus, 12/30/88).

176 068517 "Pesticide-Induced Complete and Partial Chromosome Loss in Screens with Repair-Defective Females of Drosophila melanogaster," (Woodruff et al., Environmental Mutagenesis, 5: 835-846, 1983). Diazinon was tested as a commercial pesticide preparation; the concentration of Diazinon in the preparation and the identities of the other constituents were not stated. Male flies carrying a ring X-chromosome and a doubly marked Y-chromosome were allowed to feed from a solution containing only 0.1 ppm of active ingredient (LD_{30}) for 3 days and then were mated for 3 days with mus-302 repair-defective females. Therefore, only treated mature sperm were sampled. The F. male progeny were screened for complete chromosome loss (ring X-chromosome) and partial chromosome loss (Y-chromosome marker). The use of the repair-deficient females was seen as a means to compensate for the fact that flies are inherently more sensitive to the toxic effects of insecticides. No adverse effect. Unacceptable because the test material is uncharacterized and the general idea that mating to repair-deficient females will compensate for the dose-limiting effects of

an insecticide like Diazinon in the treated males is questionable and surely requires more validation as an assay strategy. Until then, CDFA believes that Drosophila is not a system for testing insecticides, although it may be used to test pesticides less toxic to flies, like fungicides and herbicides, some of which were tested in this report at 1000-10,000 ppm. (Rinkus, 12/28/88).

153-195 085204 Rebuttal comments about why record 068510 should be downgraded presumably to "unacceptable." (Rinkus, 12/15/89).

DNA DAMAGE

Summary. This data requirement is considered satisfied based on the acceptance of records 068498, 068524, 089072, 089074, and 089075. A possible adverse effect is indicated: Diazinon is a weak inducer of sister-chromatid exchanges (SCEs) when tested in vitro. It is noteworthy that in the three positive studies, the cells or cell lines have been human in origin. In contrast to the in vitro studies, an acceptable in vivo study indicates that Diazinon does not induce SCEs in bone-marrow cells of ICR mice. (Gee, 10/5/92).

** 229 089072 "Mutagenicity Test on Diazinon MG8: In vivo Sister Chromatid Exchange Assay" (H. Murli, Hazleton Laboratories America, HLA No. 12226-0-458, 10/10/90) Diazinon MG 8, 88%, was tested in ICR mice at 0, 10, 50 or 100 mg/kg, single oral dose, 5/sex/group. Approximately 1 hour prior to dosing, a bromodeoxyuridine tablet was implanted under the skin. Animals were sacrificed approximately 25 hours after dosing and bone marrow cells harvested. Twenty-five cells in M2 were scored per animal. The average generation time was calculated from the percent of cells in M1, M2 and M3 as a measure of cell cycle delay. There was cell cycle delay at 100 mg/kg in males. No evidence for the induction of SCE's. **Acceptable** with no adverse effect. Gee, 10/1/92.

**** 229 089074** "Mutagenicity Test on Diazinon MG8 in an in vitro Cytogenetic Assay Measuring Sister Chromatid Exchange Frequencies in Cultured Whole Blood Human Lymphocytes" (H. Murli, Hazleton Laboratories America, MD, Study no. 12226-0-448, 6/25/90). Diazinon MG8, 88%, was tested with phytohemagglutinin stimulated whole blood from a single donor. Concentrations tested without activation were 0 (medium), 0 (DMSO), 0.668, 2.0, 6.68 and 20.0 Fg/ml (higher concentrations were toxic). With Aroclor-induced male rat liver S9, concentrations scored were 0, 0, 2.0, 6.68, 20 and 66.8 Fg/ml. Fifty cells in M2 per concentration were scored. SCE/cell were statistically increased without activation in trial 1 at all concentrations but not in trial 2. With activation, SSE/cell was increased at 20 and 66.8 Fg/ml in trial 1 and at all concentrations in trial 2. There was, however, considerable variability in the controls. Study is **acceptable** with a possible adverse effect. Gee, 10/2/92.

** 229 089075 "Test for the Genotoxic Effects: Autoradiographic DNA Repair Test on Rat Hepatocytes" (T. Hertner, Ciba-Geigy, Base, lab no. 891345, 2/6/90) G 24 480 technical (Diazinon), 88%, was tested for induction of unscheduled DNA synthesis with primary rat hepatocytes at 0 (DMSO), 1.1, 3.3, 10, 30, 60 or 120 Fg/ml, 16-18 hour treatment. DNA synthesis was measured by ^{3}H-thymidine incorporation followed by autoradiography. Fifty cells from each of 3 slides were scored per concentration, two trials. There was no evidence of unscheduled DNA synthesis. G 24 480 was toxic at 163.1 Fg/ml. **Acceptable** with no adverse effect. Gee, 10/2/92.

153-229 089076 "Toxicological Assessment of Diazinon: Overview of In Vitro and In Vivo Tests for DNA Damage," (D. Vlachos, Ciba-Geigy; 12/20/90). This record discusses collectively the results from records 068497, 089072, 068498, 089073, 089074 and 089075. Based on these studies, it is proposed therein that Diazinon does not cause adverse effects in the category of DNA damage. Supplementary information. No worksheet. (Rinkus, 6/1/93).

137, 146, 176 039827 "Mutagenicity Screening of Pesticides in the Microbial System," (Shirasu et al., Mutation Research, 40: 19-30, 1976), Diazinon, purity not stated, was tested in the Rec assay using B. subtilis strains H17 (Rec+) and M45 (Rec-) (details were not clearly stated). No adverse effect was reported. Unacceptable because no details or data were provided and no metabolic activation system was employed. This paper also has some other microbial testing results, e.g., Ames test and E. coli WP2 system, but this testing was only done with those pesticides that were reported to be active in the Rec assay, which therefore excluded Diazinon. (SJR 12/28/88).

176 067861 "Mutagenicity of Some Organophosphate Pesticides in Barley," (Grover, I.S. and Kaur, P., Genetics, 97: S45-S46, 1981). Abstract on the induction of chromosome aberrations and chlorophyll mutations in barley grains treated with some organophosphate pesticides, including Diazinon. No results for the Diazinon testing were mentioned. Supplemental information. No worksheet. (Rinkus, 12/28/88).

176 067601 "Cytological Effects of Some Organophosphorus Pesticides. I. Mitotic Effects," (Kaur, P. and Grover, I.S., Cytologia, 50: 187-197, 1985). Barley seeds were treated for 4 h with up to 1.0 % (v/v) Diazinon (not described) and allowed to start growing; root-tip cells were analyzed for chromosome aberrations. HOT caused 11% of the cells to have some type of aberration whereas negative controls showed a frequency of < 0.4%. Supplemental information. No worksheet. (Rinkus, 12/29/88).

176 068504 "Mutagenic Effect of a Pesticide (Ekatin) in the Soybean Test System," (Fujii, T. and Inoue, T., Environ. Exp. Botany, 23: 97-101, 1983). Soybean seeds were treated for 24 h with up to 0.2 % (v/v) Diazinon (1 to 500 dilution of an undescribed preparation) and allowed to grow; simple and compound leaves were analyzed for somatic mosaicism (spots), which would arise from a variety of genetic mechanisms (chromosome aberrations, somatic crossovers, and point mutations). No effect observed. Supplemental information. No worksheet. (Rinkus 12/29/88).

176 068514, "Chromosome Studies in Male Germinal Epithelium (Spermatocytes), G 24480, Mouse", (Ciba-Geigy Limited, Base, Switzerland; No. of experiment: 801502, Lab. report no. GU2.3, 10/20/81). G 24480, purity not stated, was administered by gavage on days 0, 2, 3, 5, and 9 at 0 (polyethylene glycol 400), 10.5, 21 or 63 mg/kg to 15 male mice/group. Mortality was 100% for the high-dose group. For each of 8 mice/treatment level, 100 spermatocyte I and 100 spermatocyte II metaphases were scored. No significant increase in aberrations was detected in spermatocyte I and II metaphases. UNACCEPTABLE, may be upgraded upon submission of evidence that assay as conducted has the sensitivity to detect gametic genotoxicants. (JSK, SJR, 12/16/88).

176 068515, "Chromosome Studies in Male Germinal Epithelium (Spermatogonia), G 24480, Mouse," (Ciba-Geigy Limited, Base, Switzerland; experiment no.801501, Lab. report no. GU 2.3, 11/6/81). G 24480, purity not stated, was administered once daily by gavage at 0 (polyethylene glycol 400), 10.5 and 21 mg/kg/day, on days 0-4, with sacrifice on day 5. For each of 8 mice/treatment level, 100 spermatogonial metaphases were scored. Spermatogonial metaphases with aberrations were not found in any of the animals studied. UNACCEPTABLE, may be upgraded upon submission of evidence that assay as conducted has the sensitivity to detect gametic genotoxicants. (JSK, SJR, 12/16/88).

176 068526 "Mutagenic, Teratogenic, and Carcinogenic Properties of Pesticides," (Durham, W.F. and Williams, C.H., Annual Rev. Entomology, 17: 123- 148, **1972**). An old review article on the mutagenicity, carcinogenicity, and teratogenicity of pesticides. Supplemental information. No worksheet. (Rinkus, 1/4/89).

176 068529 "Mutagenicity Studies on Organophosphorus Insecticides," (Wild, D., Mutation

Research, 32: 133-150, **1975**). An old review on the mutagenicity of organophosphate pesticides. Noteable for its discussion of structure-activity relationships for phosphate triesters as alkylating agents. Supplemental information. No worksheet. (Rinkus, 1/4/89).

153-192 074159 "In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides (UDS Results)," (Simmon, V., NTIS document PB80-133226). This is the EPA-sponsored study mentioned in record 068528. Diazinon, technical grade (batch FL-741305; purity not stated), was tested for the induction of unscheduled DNA synthesis (liquid scintillation counting method), using the human fibroblast cell line WI-38, in the absence and presence of a metabolic activation system (S-9 made from uninduced Swiss-Webster mouse liver). Cells that had grown to confluency were maintained in cell medium containing 0.5% fetal calf serum for 5-6 days, before being exposed to hydroxyurea. Treatment times with Diazinon (in ethanol [**Note:** a competitive substrate for P450]) were 3 h when no S-9 was employed vs. 1 h when S-9 was employed. Test concentrations (Fg/ml) were: 0 (0.5% ethanol), 0.1, 1.0, 10, and 100; precipitation was noted at the two highest treatment levels. Deficiencies in the report include: the lack of re-porting of some experimental details (e.g. passage number for cells, conversion of cpm to dpm); and the use of uninduced mouse S-9. Regarding the latter, the fact that 50 mM DMN only caused a tripling in the control values indicates that the metabolic activation system was incompetent (see also Mutation Res. 123: 363-410, 1983). Therefore, the testing of Diazinon up to 1000 Fg/ml (i.e., 3 mM) in the presence of a metabolic activation system has to be considered incomplete. In which case, the study is UNACCEPTABLE, and not upgradable, with no adverse effect indicated. (Rinkus, 1/18/90).

153-192 074159 "In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides (E. coli polA Assay and Rec Assay Results)," (Simmon, V., NTIS document PB80-133226). This is the EPA-sponsored study mentioned in records 067754 and 068528. Diazinon, technical grade (batch FL-741305; purity not stated), was tested for its ability to inhibit differentially the growth of DNA-repair proficient and deficient bacteria in the disc diffusion assay. Two strain pairs (proficient/deficient) were employed: E. coli polA (W3110/p3478) and B. subtilis rec (H17/M45). The following milligrams of Diazinon were applied to discs: 0.01, 0.1, 1.0, and 5.0. Chloramphenicol served as a negative control; without explanation, the promutagen 1-phenyl-3,3-dimethyltriazene served as a positive control..Since the testing did not include the use of any metabolic activation system, the testing is incomplete. Therefore, the study is considered UNACCEPTABLE, and not upgradable, with no adverse effect indicated. (Rinkus, 1/18/90).

153-192 074159 "In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides (S. cerevisiae Results)," (Simmon, V., NTIS document PB80-133226). This is the EPA-sponsored study mentioned in records 067754 and 068528. Diazinon, technical grade (batch FL-741305; purity not stated), was tested for the induction of recessive homozygosity in the heterozygous diploid yeast strain S. cerevisiae D3; in this strain, this could result from one or more of the following genetic events: reciprocal recombination, gene conversion, point mutation, deletion, and chromosome loss. Testing was performed in the absence and presence of a metabolic activation system (S-9 made from Aroclor 1254-induced male SD rat liver). The following concentrations (% v/v) were prepared in DMSO: 0 (DMSO), 0.1, 0.5, 1.0, and 5.0 for the first trial; 0, 1.0, 2.0, 4.0, and 5.0 for the second trial; and 0, 1.0, 3.0, 4.0, and 5.0 for the third trial. Testing involved adding 0.2 ml of the DMSO solution to 1.8 ml of a mixture of the yeast culture and either phosphate buffer or S-9 mix. Deficiencies in the report include: the lack of reporting on some experimental details, testing with only stationary-phase cells, the lack of testing up to toxicity (by longer incubation times since maximum solubility had been exceeded even by the lowest concentration tested [Mutation Res. 133: 199-244, 1984]); and the failure to use a promutagen as a positive control for the metabolic activation system. This study is considered UNACCEPTABLE, and not upgradable, with no adverse effect indicated. (Rinkus, 1/18/90).

178 068497 "Diazinon--Tests for Other Genotoxic Effects: Sister Chromatid Exchange Study", (Ciba-Geigy Limited, Base, Switzerland; Lab. study no. 801504, 10/13/81). G 24480 (Diazinon), purity not stated, was given once by gavage at 0 (PEG 400), 6.5, 13 or 26 mg/kg to Chinese hamsters, 1-2/sex/group; animals were sacrificed 24 h after dosing. The frequency of SEES was not increased statistically when the pooled male and female data were analyzed. However, the mid and high doses did increase the SSE frequency in the females but the statistical significance could not be assessed because there was only one female in the vehicle control group. **UNACCEPTABLE** and not upgradeable because too few animals were used and the testing was not done up to the M.D.. (Kishiyama & Rinkus, 12/14/88).

**** 178, 229 068498, 089073** "Diazinon--Tests for Other Genotoxic Effects: Sister Chromatid Exchange Test on Human Lymphocytes Cell Line CCL 156 in vitro" (F. Strasser, Ciba-Geigy Limited, Base, Switzerland, Lab. No. 871697, 5/16/88 and 6/26/90). G 24480 Technical, 87.5% purity, with and without S9, was tested at 0 (1% DMSO as final concentration in the cell medium), 12.5, 25, 50, 100 or 200 Fg/ml for induction of sister-chromatid exchanges in human lymphoid cell CCL 156. Cells were treated for 3 hours. Possible adverse effect. In the presence of S9, four of five treatment levels increased statistically ($p<0.05$) the SSE frequency. In the absence of S9, the two lower concentrations increased statistically ($p<0.01$) the SSE frequency. In both cases, linear dose responses were not evident. This study was considered in the original review as acceptable (Kishiyama and Rinkus, 12/8/88). In record 085205, the registrant discussed that since a doubling of the SSE frequency was not observed, the study should be considered as indicating that diazinon does not induce SSE's. DPR Medical Toxicology maintained that the study was acceptable and indicated that diazinon is a weak inducer of SSE's and that only retesting or submission of historical control data and cell cycle frequency data would be useful. (Rinkus, 1/12/90). Record 089073 contains baseline data from two additional studies with CCL 156 conducted in 1987/1988. The results with diazinon and CCL 156 are now considered equivocal. The study remains **accept-able** with a possible adverse effect. Gee, 10/2/92.

153-195 085205 Rebuttal comments about why record 068498 should be downgraded presumably to "unacceptable." (Rinkus, 1/12/90).

176 068519 "Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Organophosphate Insecticides and their Oxygen Analogs," (Nishio, A. and Uyeki, E.M., J. Toxicology Environ. Health, 8: 939-946, 1981). Diazinon, 89% purity, was tested at 0 (0.5% DMSO final concentration), 0.03 , 0.1, 0.3, and 1.0 mM (0, 9.1, 30.4, 91.3, and 304.4 Fg/ml, respectively) without any metabolic activation, for its ability to increase the frequency of SEES in Chinese hamster ovary cells. Diazoxon, the oxygen analog of Diazinon, also was tested up to 1 mM. Treatment time with Diazinon and Diazoxon was 38 h and 20-50 metaphases/treatment level were scored in each case. No SSE enhancement and no inhibition of the cell cycle was noted even at the HOT for Diazinon. However, Diazoxon increased the mean number of SEES per cell from 7.1 in the controls to 9.6 at the 1.0 mM treatment level ($p\leq0.01$); but like Diazinon, Diazoxon did not inhibit the cell cycle even at the HOT. Unacceptable because the testing did not use any metabolic activation system; also, the lack of cytotoxicity or cell-cycle effects at the high treatment levels is not consis-tent with the observation of such effects in other studies of Diazinon. (Rinkus, 1/3/89).

176 068520 "Sister Chromatid Exchanges in Chinese Hamster Cells Treated with Seventeen Organophosphorous Compounds in the Presence of a Metabolic Activation System," (Chen et al., Environmental Mutagenesis, 4: 621-624, 1982). Diazinon, 99.2% purity, was added to diffusion chambers containing a rat-liver S-9 (not described) and then the diffusion chambers were placed in culture flasks (volume=20 ml) containing Chinese hamster V79 cells. The treatment levels, expressed as Fg per ml of medium, were: 0 (DMSO, but final concentration in the medium after diffusing out was not stated), 10, 20, 40, and 80; however, it is questionable whether Diazinon diffused

out of the chambers such that these concentrations actually were established. Cells were treat-ed for 25-27 h and a total of 50 cells were analyzed for SEES at each treatment level. No toxicity was mentioned, which is crucial in this case since the test material is being added to diffusion chambers that contain the S-9 mix and, therefore, its lipids and protein may bind the test material. No adverse effects noted. Unacceptable because Diazinon was not tested up to toxicity; also, it is questionable whether short-lived metabolites would be able to diffuse out of the chambers into the media containing the cells. (Rinkus, 1/3/89).

176 068521 "Induction of Sister-Chromatid Exchanges and Cell Cycle Delay in Cultured Mammalian Cells Treated with Eight Organophosphorus Pesticides," (Chen et al., Mutation Research, 88: 307-316, 1981). Diazinon, 99.2% purity, was tested at 0 (DMSO but its final concentration in the medium was not stated), 10, 20, 40, and 80 Fg/ml, without any metabolic activation system, for its ability to increase the frequency of SEES in Chinese hamster V79 cells. The treatment period was 29 h; generally, 50 cells/treatment level were analyzed. Three experiments covering the range of 0 - 40 or 0 - 80 Fg/ml were conducted. In one experiment, 80 Fg/ml caused so much cell cycle delay that the SSE analysis could not be performed. Otherwise, no adverse effect was noted in three experiments that tested up to 40 Fg/ml. Unacceptable because testing did not include the use of a metabolic-activation system. (Rinkus, 1/3/89).

176 068522 "In Vivo Induction of Sister-Chromatid Exchange in Umbra limi by the Insecticides En-drin, Chlordane, Diazinon and Guthion," (Vigfusson et al., Mutation Research, 118: 61-68, 1983). Mudminnows (Umbra limi) were exposed in their aquaria for 11 days to Diazinon, only **49% purity**, at final concentrations in the water of 54, 540, and 5400 picomolar (0.016, 0.16, and 1.6 ng/ml, re-spectively); the intestines were removed and 25 cells/fish were scored for SEES. The HOT was toxic to the fish (12 of 15 fish died by day 11) and the frequency of SEES was increased by a fact-or of three over the controls; the mid dose was only slightly toxic and the frequency of SEES was increased by a factor of two; the low dose was without effect. Supplemental information. No work-sheet. (Rinkus, 1/3/89).

****153-176 068524** "Cytokinetic and Cytogenetic Effects of Some Agricultural Chemicals on Human Lymphoid Cells In Vitro: Organophosphates," (Sobti et al., Mutation Research, 102: 89-102, 1982). Diazinon, purity not stated, was tested at 0 (0.1% (v/v) ethanol as final concentration), 0.02, 0.2, 2.0, and 20 Fg/ml, without metabolic activation, and at 20 Fg/ml, with metabolic activation, for its ability to increase the frequency of SEES in the human lymphoid cells LAZ-007. Metabolic activation involved the use of a phenobarbital-induced rat-liver S-9 product obtained commercially. Without S-9, cells were treated for 48 h; but with S-9, cells were treated for only one hour. Without S-9, the 2 and 20 Fg/ml treatments resulted in cell-cycle delay and the latter treatment also gave the maximum increase in the mean SSE frequency, from 6.96 in the controls to 8.60; this difference was reported as not significant at the 0.01 level, the only level of significance considered in the report. In a second experiment, the 20 Fg/ml treatment was repeated, without and with S-9 activation, although the treatment time for both apparently was only one hour. With-out S-9, the mean SSE frequency increased from 6.87 in the controls (0.1% ethanol) to 7.80. With S-9, the mean SSE frequency increased from 6.79 in the controls (0.1% ethanol) to 13.50; this was reported as significant at the 0.01 level in comparison to the mean SSE frequency of 7.80 when S-9 was not included in the treatment (this was the only statistical comparison reported). This study was considered in its original review as ACCEPTABLE and indicating an ADVERSE EFFECT (Rinkus, 1/4/89). In record 085206, the Registrant argued that the experiment needed to be repeated in order to be acceptable and, therefore, the study should be considered only as supplemental information. However, CDFA is persuaded from the good reproducibility among four negative controls that the enhanced SSE frequency observed with Diazinon in the presence of the metabolic activation system is unlikely to be due to chance. (Rinkus, 1/12/90).

153-195 085206 Rebuttal comments about why record 068524 should be downgraded presumably to "unacceptable." (Rinkus, 1/12/90).

176 068525 "Intercambio de Cromatidas Hermanas en Trabajadores de una Industria Quimica," (Gonzalez Cid, M.B. and Matos, E., Medicina (Buenos Aires), 43: 513-516, 1983). Spanish article with English summary, concerning a SSE study of workers at a chemical plant that manufactures several pesticides, including Diazinon: negative results were reported. Supplemental information. No worksheet. (Rinkus, 1/4/89).

NEUROTOXICITY, HEN

151 039839 "Diazinon: Demyelination Study in the Chicken (Diazinon 50W)."(Woodard, 1964) Diazinon, 48.6%, was given at 0, 2, 20 or 200 ppm in the diet for 7 weeks (severe toxicity in 200 ppm group limited dosing period for that group to 3 weeks). No delayed neuropathy symptoms reported at 200 ppm diazinon. TOCP group had classic delayed response. **Unacceptable.** Missing information, non-standard protocol. J. Parker and J. Gee, 3/13/86.
EPA One-liner: No core grade. NOEL = 200 ppm (HOT).

**178 068500 "Acute Delayed Neurotoxicity of Diazinon MG-8 in Domestic Fowl," (Stillmeadow, Inc., Houston, Texas; Lab. study no. 5152-87, 5/23/88). Diazinon MG-8, purity 87%, was given by gavage on day 0 at 28.1 mg/kg to 18 domestic chicken hens. Due to a lack of effects, a second dosing was done on day 21, using 13.8 mg/kg. (The first dosing at 28.1 mg/kg was accidental and resulted from misformulating the dosing solution; the second dosing was done at the intended level, 13 mg/kg). The unprotected LD50 of Diazinon MG-8 was 12.5 mg/kg. There were no signs of delayed neurotoxicity and neural tissues were unremarkable on microscopic examination. No adverse effect noted. Acceptable. (JSK, SJR, 12/16/88).

NEUROTOXICITY, RAT

**153-363 136804 "Acute Neurotoxicity Study with DZN® Diazinon MG87% in Rats" (Chow, E. & Richter, A.G.; Plant Protection Division, Ciba-Geigy Corp., Farmington, CT; laboratory study no.: F-00175; 1/20/94). Diazinon (88% purity) was administered by gavage one time to 15 Hsd: Sprague Dawley SD rats/sex/dose level. The dosing volume was 5 ml/kg. Dose levels were: 0 (corn oil), 2.5, 150, 300 and 600 mg/kg. A sixth group, consisting of 10 rats/sex, was gavaged one time with a corn oil solution containing Triadimefon to achieve a dose of 150 mg/kg; this group served as a positive control (Neurotoxic. Teratology 11:285-293, 1989; Fund. Appl. Toxicol. 10: 459-465, 1988). Diazinon dose levels were selected based on the results from a range-finding study (record 136807). Neurobehavioral testing using 10 rats/sex/dose was performed four times: pre-exposure; at the estimated time of peak effect (9-11 h and 1 h postdosing for Diazinon and Triadimefon, respectively; hereafter referred to as their peak times); ~7 d postdosing and ~14 d postdosing. Testing included a Functional Observation Battery (FOB) and automated assessments of motor activity in figure-8 mazes. Rats used in the FOB testing underwent perfusion fixation before being necropsied. In 5 rats/sex from the negative control groups, the 600 mg/kg Diazinon groups and the Triadimefon groups, selected tissues were examined microscopically. Cage-side findings in both sexes on study days 1 or 2 included: reduced activity (LOEL= 300 mg/kg [males]); tremors; chromodacryorrhea; chromorhinorrhea; and pallor. Weekly bodyweight measurements were not affected whereas FOB bodyweights measured at 9-11 h postdosing were statistically decreased for the groups (both sexes) dosed with Diazinon at $\geq$ 300 mg/kg. Each of the five "functional categories" of the FOB testing had endpoints which were affected by Diazinon; these effects were seen only at the peak-time testing. Autonomic effects included: partially formed fecal pellets (LOEL = 2.5 mg/kg [males]); impaired

respiration; increased lacrimation; increased salivation (males only); soiled fur; chromorhinorrhea; and chromodacryorrhea. Effects on muscle tone and equilibrium included: ataxic or abnormal gait (LOEL = 2.5 mg/kg [both sexes]); abnormal righting reflex; abnormal hind-limb extension; decreased forelimb and hindlimb grip strengths; body twitch; and muscle fasciculations. The incidence of no reaction to tail pinching (a sensorimotor response) was increased in both sexes at 600 mg/kg. Endpoints concerning CNS activity and excitability that were affected included: reduced rearing activity (LOEL = 150 mg/kg [females]); reduced level of arousal; increased ease of handling; increased ease of removing from the cage; tremors; and the appearance of a stereotypic behavior involving opening and closing the mouth (LOEL = 150 mg/kg [females]). Physiological effects included: decreased bodyweight at peak time; and decreased rectal temperature (LOEL = 150 mg/kg [females]). Although dehydration was noted at high incidence in the 300 mg/kg and 600 mg/kg female groups, whether this constitutes a true female-specific effect is unclear, for reasons discussed in the worksheet W136804.818. Motor activity assayed at peak time was decreased by Diazinon, with LOELs of 150 mg/kg in the females and 300 mg/kg in the males. Inhibition of cholinesterase activity was seen at peak time, with LOELs of 2.5 mg/kg (serum, both sexes) and 150 mg/kg (RBC, both sexes). Only inhibition of RBC cholinesterase was seen in the testing done ~2 weeks postdosing; the LOELs were 150 mg/kg (females) and 300 mg/kg (males). Brain cholinesterase activity, which was only assayed ~2 weeks postdosing, was not affected. No effects were noted at necropsy or during the histological examination. With the exception of increases in the arousal level and in the incidence of rearing at 1 h postdosing in the males, the Triadimefon treatment did not fulfill its function as a positive control for the FOB testing. In motor activity testing, Triadimefon did produce an unequivocal in-crease in motor activity in both sexes when assayed at its peak time. **Acute Neurotoxicity NOEL < 2.5 mg/kg (altered gait; partially formed feces); Overall Neurotoxicity NOAEL > 600 mg/kg (acute effects not present at 1 or 2 weeks postdosing).** When first reviewed (8/15/96), this study was considered unacceptable pending submission of positive-control data for FOB testing, motor-activity testing and nervous-system-pathology assessments and supplemental information regarding dehydration in the females. The requested supplemental information was supplied in re-cords 155834 through 155842. For the reasons discussed in worksheet w136804.s01, this study is now considered marginally **ACCEPTABLE**. (Rinkus, 6/30/98).

153-366 136807 "Acute Rangefinding Neurotoxicity Study with DCZCN® Diazinon MG87% in Rats," (C.L. Leahy; Plant Protection Division, Ciba-Geigy Corp., Farmington, CT; laboratory study no.: F-00174; 11/9/93). Hsd:Sprague Dawley SD rats (Harlan, Indianapolis, IN; 34 days old; both sexes) were gavaged once with Diazinon (88% purity) after being fasted for ~12 h and then were evaluated for neurological effects using an abbreviated FOB (hereafter referred to as AFOB). The intent was to determine the time(s) at which the neurological effects produced by Diazinon were maximally seen (so called time of peak effect). Six rats/sex/dose were tested in the AFOB. The evaluating was done using two sets of three rats: one set of three rats was evaluated at 3, 6, 9 and 12 h postdosing while the second set was evaluated at 15, 18, 21 and 24 h postdosing. Mortality checks were made for 7 days postdosing. Males were dosed at: 0 (corn oil), 250, 500, 750 and 1400 mg/kg; and females were dosed at: 0 (corn oil), 500, 750, 1000 and 1400 mg/kg. In both sexes dosed at 1400 mg/kg, death occurred within 24 h. Since such lethality is not consistent with the oral LD50 values of 1380 mg/kg for males and 1150 (and 1260) mg/kg for females cited in record 136807, this may indicate a greater sensitivity for the 34-day old rats vis-a-vis the ~8-week old rats conventionally used in acute studies. Three other deaths occurred in the study: two deaths out of the 6 females dosed at 1000 mg/kg (time of death not stated); and one death out of the 6 females dosed at 750 mg/kg (time of death not stated). Neurological effects included: autonomic effects (e.g., partially formed feces/diarrhea, salivation, lacrimation and impaired respiration); effects on muscle tone and equilibrium (e.g., abnormal and ataxic gait, abnormal righting reflex and muscle fasciculations); and effects on CNS activity and excitability (e.g., reduced activity, tremors and a stereotypic behavior involving opening and closing the mouth). These effects were seen at the respective lowest doses

tested for each sex (250 mg/kg in the males and 500 mg/kg in the females). For the males in the lowest dose group (250 mg/kg), neurological signs exhibited a peak effect at 6 h postdosing whereas dosing at higher levels was associated with peak times that increased to as much as 11 h in the 750 mg/kg male group. Recovery was evident between 9 and 21 h post-dosing for males dosed at 250 mg/kg but neurological signs persisted up to 24 h in the males in the higher dose groups. **Supplemental information. No worksheet.** (Rinkus, 8/20/96).

153-398 136901 "Acute Cholinesterase Inhibition Time Course Study with DCZON® Diazinon MG87% in Rats," (R.F. Potrepka; Plant Protection Division, Ciba-Geigy Corp., Farmington, CT; laboratory study no.: F-00185; 1/12/94). Hsd:Sprague Dawley SD rats (Harlan, Frederick, MD; 7 weeks old; both sexes) were gavaged one time with Diazinon (88% purity) after being fasted for ~12 h. Doses were the same as used in record 136804, i.e., 0 (corn oil), 2.5, 150, 300 and 600 mg/kg. Rats were bled for determination of serum and RBC cholinesterase activities and subsequently were sacrificed for determination of cholinesterase activities in the following: cerebellum, cerebral cortex, striatum, hippocampus and thoracic spinal cord. For each sampling time, five rats/sex/dose were used. Sampling times were: 3 h (a prepeak-effect time), 9 h (peak effect time) and 24 h postdosing (a postpeak-effect time). The intent was to determine the time course of cholinesterase inhibition. There was no FOB testing of any type and no bodyweight measurements. Cageside observations were recorded immediately before sampling; as a result, the number of rats/sex/dose/sampling time was not 15, but rather only five. No deaths were seen during the 24 h following the dosing. One or more of the following clinical signs were noted at 3 h postdosing in three of the five 600 mg/kg males (unless noted otherwise): chromodacryorrhea, chromo rhinorrhea (two of the five 600 mg/kg females also affected), loose stool and muscle fasciculations. At 9 h postdosing, each of the five males/group in the 300 mg/kg and 600 mg/kg groups, two of the five females in the 300 mg/kg group and each of the five females in the 600 mg/kg group exhibited one or more of the aforementioned clinical signs; also, salivation and reduced activity were noted in some 600 mg/kg males. At 24 h postdosing, the 300 mg/kg groups (both sexes) were essentially without clinical signs while all rats (both sexes) dosed at 600 mg/kg continued to show clinical signs. Cholinesterase activity measurements indicated the following LOELs for cholinesterase inhibition: serum, 2.5 mg/kg (both sexes, at each of the sampling times); and RBC, 150 mg/kg (males, at each of the sampling times; and females, at 3 and 24 h postdosing) vs. 2.5 mg/kg (females, at 9 h postdosing only). For the regional CNS cholinesterase measurements, the lowest LOEL was 2.5 mg/kg; it was seen with the cerebral cortex of the males at 9 h postdosing. Other-wise, for the other regions, 150 mg/kg was the LOEL; this was true for both sexes at each sampling time. In the males dosed at 150 mg/kg, the maximal inhibition for each region tended to be seen at 9 h postdosing, with some recovery (less inhibition) being seen at 24 h postdosing. How-ever, in the females dosed at 150 mg/kg, the maximal inhibition for several regions was seen at 9 h as well as 24 h postdosing (i.e., no recovery was evident at 24 h postdosing, as seen with the males). **Supplemental information. No worksheet.** (Rinkus, 8/20/96).

****153-313 135600** "90-Day Subchronic Neurotoxicity Study with DZN® Diazinon MG87% in Rats" (Pettersen, J.C. & Morrissey, R.L.; Crop Protection Division, Ciba-Geigy Corp., Farmington, CT; laboratory study number: F-00176; 8/26/94). Diazinon (88% purity) was administered to 15 Crl:CD®(SD) BR rats/sex/dose level in the diet at 0 ppm, 0.3 ppm, 30 ppm, 300 ppm and 3000 ppm for 7 d/week, for a total of 13 weeks. At week 13, this resulted in Diazinon dose levels of 0, 0.012, 1.2, 12 and 142 mg/kg-d for the respective male groups and 0, 0.015, 1.5, 15 and 178 mg/kg-d for the respective female groups. Dose levels had been selected on the basis of studies conducted previously (records 139303 & 092996); also, an acute neurotoxicity study (record 136804) and a 28-day neurotoxicity study (record 135599) have been submitted. Neurobehavioral testing involved 10 rats/sex/dose group and was done preexposure and during study weeks 4, 8 and 13. Testing included a Functional Observation Battery (FOB) and automated assessments of motor activity in figure-8 mazes. Rats used in the FOB testing underwent perfusion fixation; these rats were necropsied and selected tissues were examined microscopically. Cageside findings were limited to

the following: muscle fasciculations in 8 females and tremors in two females in the 3000 ppm group; and hyperresponsiveness in two males in the 3000 ppm group. In ophthalmological examinations done at the end of the study, dry corneas were noted bilaterally in two 3000 ppm females. The bodyweights of the male and female 3000 ppm groups were statistically reduced, starting with week 1 and lasting till week 6 (males) or week 11 (females). However, review of the bodyweight change data and the feed consumption data suggests that the bodyweight reduction may have been at least partially due to a palatability problem (i.e., a reduction in feed consumption in the first weeks of the study was noted). Statistically significant FOB findings were seen only with the 3000 ppm female group; they included: decreased forelimb grip strength (weeks 4 through 13); decreased hindlimb grip strength (week 4); and decreased rectal temperature (week 13). A statistically significant decrease in hindlimb foot splay at week 4 in the 3000 ppm female group was made questionable by the occurrence of outliars in the controls and dehydrated animals in the 3000 ppm female group. While no statistically significant effects were noted in the FOB testing of the males, decreases in forelimb and hindlimb grip strength were seen with the 3000 ppm group. Muscle fasciculations after forelimb and hindlimb grip strength testing were seen in two 3000 ppm males (weeks 4 & 13), in one 300 ppm male (week 13), and in the majority of the 3000 ppm females at weeks 8 & 13. Motor activity testing did not identify any treatment-related effects for either sex. Cholinesterase activity measurements from 5/sex/group for the serum and red blood cells indicated NOELs of 0.3 ppm (both sexes; weeks 4 through 13). Cerebral cortex/hippocampus cholinesterase activity measurements done in week 13 indicated NOELs of 0.3 ppm in the females vs. 300 ppm in the males. The cholinesterase activities measured in the cerebral cortex/hippocampus of the 3000 ppm females and the 300 ppm females were 8% and 25%, respectively, of the level measured in the female controls; the 3000 ppm males exhibited 23% of the level measured in the male controls. No necropsy or histological findings were reported. **Neurotoxicity NOAEL = 0.3 ppm (brain cholinesterase inhibition in the females at week 13).** When first reviewed (6/18/96), this study was considered unacceptable and upgrading required the submission of positive control data for FOB testing, motor-activity testing and neuropathology and supplemental information concerning the following: dehydration; anomalous bodyweight loss; how the clinical data were recorded; ophthalmology examinations; and historical control data for dry corneas. The re-quested supplemental information was supplied in records 155834 through 155842. For the reasons discussed in worksheet w135600.s01, this study is now considered marginally **ACCEPT-ABLE**. (Rinkus, 7/17/98).

153-312 135599 "Cholinesterase Inhibition in 28 Day Feeding Study in Rats," (J.C.F. Chang; Crop Protection Division, Ciba-Geigy Corp., Farmington, CT; laboratory study number: F-00186; 11/7/94). Fifteen rats/sex/dose level were administered Diazinon (88% purity) in their diet at the same levels used in record 135600, i.e., 0 ppm, 0.3 ppm, 30 ppm, 300 ppm and 3000 ppm. The rats (Crl:CD BR; Charles River, Raleigh, NC) were ~7 weeks old at the start of the exposures. Five rats/sex/dose level were sacrificed at the end of study weeks 1, 2 and 4 weeks for the purpose of determining serum, RBC and regional CNS cholinesterase activities. There was no neurobehavioral testing of any type. Cageside observations were recorded twice daily for all rats on test. Weekly bodyweight and food consumption measurements also were done. No deaths were seen during the study. Clinical signs, bodyweight effects and food consumption effects resulting from exposure to Diazinon were limited to the 3000 ppm groups. Females appeared to be more affect-ed than the males. Muscle fasciculations involving the forefeet were seen with three males, starting day 8: two of the affected males were sacrificed that same day (by experimental design) while the other continued to be affected until sacrificed on day 29. By contrast, 13 of the 15 females in the 3000 ppm group exhibited muscle fasciculations during the study, with 8 of the 13 exhibiting them starting day 8 (note: also by experimental design, some of these affected females were sacrificed on day 8 [or day 15]). Generalized muscle fasciculations, as opposed to those involving the forefeet only, were noted with 8 of the 13 affected 3000 ppm females; diarrhea also was noted in three of the 13 affected females. Food consumption by the male and female 3000 ppm groups was reduced to 78% and 73% of that

seen with the respective control groups in study week 1; afterwards, there was little if any effect. Therefore, the bodyweight gain differences between the 3000 ppm groups (both sexes) and their respective control groups, which were evident after one week of exposure, may be partly--if not entirely--due to this palatability problem, as opposed to being due to toxicity. Cholinesterase activity measurements indicated the following LOELs for cholinesterase inhibition: serum, 0.3 ppm (males, weeks 1 and 2) vs. 30 ppm (females, all time points); RBC, 30 ppm (both sexes, all time points); cerebellum, 300 ppm (both sexes, all time points); cerebral cortex, 3000 ppm (males, all time points) vs. 300 ppm (females, all time points); hippocampus, 3000 ppm (males, all time points) vs. 300 ppm (females, all time points); striatum, 300 ppm (males, weeks 1 and 2; and females, all time points); and thoracic spinal cord, 3000 ppm (males, all time points) vs. 300 ppm (females, all time points). A high level of brain cholinesterase inhibition was seen in the 3000 ppm rats, especially the females (e.g., at week 4, regional CNS cholinesterase activities were 3-11% of the respective control values). **Supplemental information. No worksheet.** (Rinkus, 8/20/96).

SUPPLEMENTAL STUDIES

179 068501 "Diazinon Technical: 21-Day Dermal Toxicity Study in Rabbits", (Ciba-Geigy Corporation, Pharmaceuticals Division, Summit, NJ; Lab. study No. 842007, 6/11/84). Diazinon techni-cal, 97.1% purity, was applied topically at 0 (50% aqueous polyethylene glycol 300), 1, 5 or 100 mg/kg/day to New Zealand White rabbits, 5/sex/group, 5 days/week, for 3 weeks. On day 8, the HDT was lowered to 50 mg/kg/day due to four deaths among the high-dose males. Clinical signs of organophosphate intoxication were only seen in the high-dose animals. The kidney/body weight ratio was decreased by 28% in the high-dose females. Brain cholinesterase was decreased by 43% and 18% in the high- and mid-dose females, respectively. Serum cholinesterase was de-creased by 57%, 18% and 14% (relative to predosing values) in the high-, mid-, and low-dose fe-males, respectively. RBC cholinesterase was decreased by 13% (relative to predosing value) in the high-dose females. ChE NOEL = 1 mg/kg/day (brain and serum cholinesterase inhibition in females). Major deficiencies include: 1) dosing solutions were not analyzed for content, homogen-eity, or the stability of Diazinon in what were reported to be mildly acidic suspensions; and 2) the dose selection was not useful for

understanding the noncholinesterase effects of Diazinon. Sup-plemental information. (JSK, SJR, 12/20/88).

177 068532 "Effects of Insecticide, Diazinon, on Pancreas of Dog, Cat, and Guinea Pig." (Frick et al., J. Environ. Path. Toxicol. Oncol., 7:1-12, 1987) Article presents evidence that Diazinon inhibi-tion of pancreatic butylcholinesterase (BuChE) leads to cholinergic hyperstimulation of the acinar cell, which results in acute pancreatitis in dogs and guinea pigs. Diazinon did not induce compar-able pathological effects in cats because, unlike dogs and guinea pigs, cats lack pancreatic BuChE. Article also notes that pancreatitis in humans has been reported after accidental expo-sure. Supplemental information. No worksheet. (Rinkus, 2/22/89).

177 068554 "Ocular Symptoms Induced by Organic Phosphorous Insecticides." (Kogure and Imai, J. American Med. Women's Assoc.,30:420-422, 1975). An old article that is notable for its review of "Saku disease," which is an induction of visual disorders due to chronic exposure to organo-phosphate insecticides. Saku disease takes its name from the Saku district of Nagano Prefect in Japan; the visual complications were originally observed in children of this district. Supplemental information. No worksheet. (Rinkus, 2/22/89).